IMMUNOLOGICAL STUDIES OF THE PROTEIN MOIETIES OF HUMAN PLASMA VERY LOW-DENSITY AND LOW-DENSITY LIPOPROTEINS

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Received 21 December 1973

1. Introduction

We have studied by immunological methods, native and chemically modified LDL*, and the relationship of its protein moiety, Apo B, with the major protein components of VLDL, Apo B and Apo C. The results reported concerned 5 to 10 different normal lipoprotein preparations whose purity had been previously controlled by immunodiffusion and immunoelectrophoresis, with several specific and heterologous antisera.

2. Materials and methods

Ultracentrifugation was performed according to Havel and al. [1] at density 1006 for VLDL and between 1.024–1.045 for LDL. The covalent modification of LDL was achieved with maleic anhydride in 100-fold molar excess over lysine residues, by the technique of Butler et al. [2]. Residual maleate was removed by extensive diafiltration on Diaflo-membrane UM-2.

For the preparation of apoproteins, maleyl LDL was delipidated by ethanol ether 3:1; and the precipitated protein solubilized in Tris buffer pH 8.0 with and without sodium dodecyl sulfate 0.2 M final concentration. Partial delipidation was obtained with ether alone; in these conditions apoprotein preserved its solubility. Apoproteins C and B of VLDL were prepared as follows:

– Gel filtration on Sephadex G-150 in SDS buffer of Apo-VLDL according to Brown and al. [3].

– The apo B fraction of VLDL was selectively extracted using Kane's technique [4] with tetramethyl urea v/v. After centrifugation 10', 3000 rpm, the floating "cake" containing the B fraction was washed twice with TMU, delipidated with ethanol ether 1:3 and then dissolved in a Tris–SDS buffer. The TMU soluble fraction (apo C) was recovered by alcohol precipitation, dissolved in Tris buffer and any precipitating material discarded after centrifugation.

Immunodiffusion [5] and immunoelectrophoresis [6] were carried out in 1% agarose in veronal buffer pH 8.2. Amido-black and Sudan-black were used for protein and lipid staining. All precipitation reactions were carried out in antibody excess. For the passive microhaemagglutination rabbit red cells were coated with antigens by glutaraldehyde as described by Avrameas [7]. Antisera against LDL, Maleyl LDL, Maleyl Apo B, VLDL, Apo B of VLDL and Apo C, were prepared by injecting several rabbits with 200 μg protein antigen and 0.15 ml complete Freund's adjuvant, twice at three weekly intervals. The inoculations were intradermally administered in several spots in the back.

3. Results

In IEP against an anti LDL serum, Maleyl LDL pre-

* Abbreviations used are: LDL: low density lipoprotein; VLDL: very low density lipoprotein; Apo B: apoprotein B; Apo C: apoprotein C; ID: immunodiffusion; IEP: immunoelectrophoresis; Mal-LDL: maleated low density lipoprotein; SDS: sodium dodecyl sulfate; TMU: tetramethyl urea.

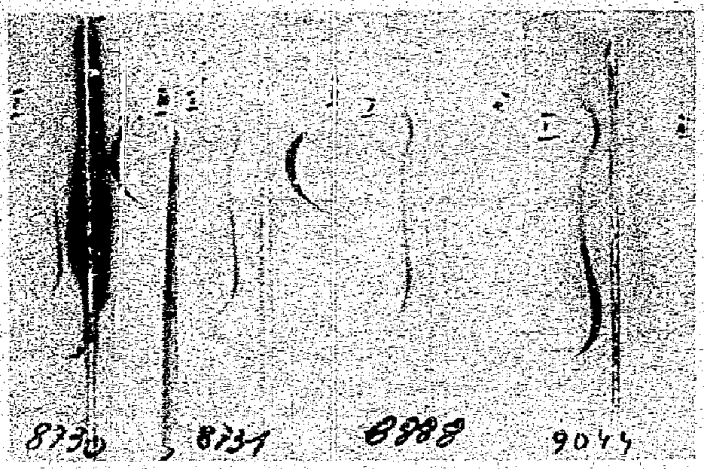

Fig. 1. IEP of native and modified LDL against an anti LDL serum. Slide 1 (Amido black staining): I. Mal. LDL II. LDL; Slide 2 (Sudan black staining): I. Mal. LDL II. LDL; Slide 3 (Amido black staining): I. Mal. LDL II. Partially delipidated Mal. LDL; Slide 4 (Amido black staining); I. Mal. LDL II. Mal. Apo. B.

sented a good reactivity (protein and lipid staining). Maleyl Apo B did not react whereas partially delipidated maleyl LDL (about 60% delipidation for phospholipids and 95% for cholesterol) still reacted. The increased anodal mobility of the modified proteins can be explained by their high anoinic charge (fig. 1). The same results were obtained by immunodiffusion (fig. 2).

The loss of reactivity of maleyl Apo B is restored in presence of SDS: maleyl Apo B, as shown in fig. 3, does not react, whereas the same product solubilized

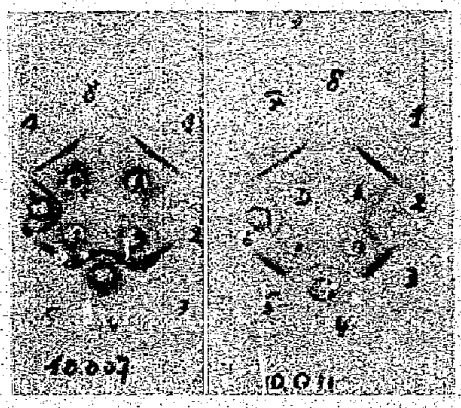

Fig. 2. ID of native and modified LDL against an anti LDL serum (Amido black and Sudan black staining). 1, 3, 5, 7, LDL; 2, Mal. LDL; 4, 6, partially delipidated Mal LDL; 8, Apo B.

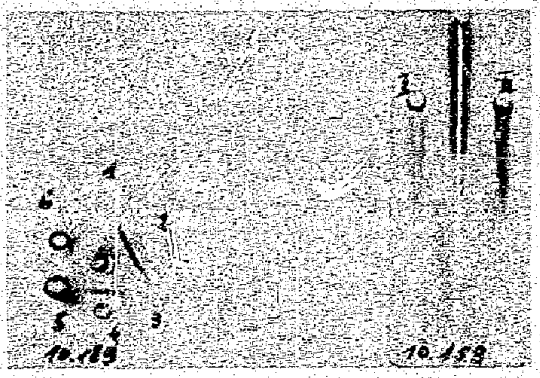

Fig. 3. ID and IEP of LDL and modified LDL with an antiserum against partially delipidated LDL (Amido black staining). ID: 1 Mal. LDL; 2, LDL; 3, Mal. Apo B; 4, partially delipidated Mal. LDL; 5, Apo B of VLDL in SDS; 6, Mal Apo B in SDS; IEP: I, Mal. Apo B in SDS; II Apo B of VLDL in SDS.

in presence of SDS does (well 6). In IEP with an antiserum against partially delipidated LDL, maleyl Apo B and VLDL Apo B solubilized in SDS, exhibit several precipitation lines (fig. 3). No precipitation lines were found when SDS-solubilized apoproteins B were submitted to reaction with several nonspecific rabbit antisera.

The rabbit antisera we obtained indicated that all the products we used for immunization were good immunogens.

Antiserum against maleyl LDL reacted strongly with maleyl LDL, and less with native LDL, showing at the same time a partial relationship. Antiserum against maleyl Apo B reacted with maleyl LDL, maleyl Apo B and although weaker, also with native LDL.

Fig. 4 shows the reactions obtained with antisera against Apo B of VLDL and Apo C. VLDL Apo B antiserum reacts strongly with VLDL Apo B and maleyl LDL, and unexpectedly, also with Apo C. A partial relationship and a spur are seen between Apo C on one side, and maleyl LDL and Apo B on the other. Apo C antiserum, presented two precipitation lines in reacting with Apo C; one of these, the inner line, shows an identity reaction with maleyl Apo B.

Furthermore passive microhaemagglutination, confirmed the positive reactions previously mentioned (table 1).

Moreover, maleyl Apo B which reacted in ID only with homologous antiserum agglutinated in presence of all the related antisera under study.

Table 1
Comparison between gel immuno-precipitation and passive micro-haemagglutination

Antisera \ Antigens		LDL	Maleyl LDL	Maleyl apo-B	Maleyl LDL partial delipidation	Maleyl apo-B in SDS	VLDL	VLDL apo-B	VLDL apo-B in SDS	VLDL apo-C
Total human AS	IEP, ID	++	+	–	+	+	++	–	+	–
	PH	++	++	+	++		++	++		+
LDL AS.	IEP, ID	++	+	–	+	+	++	–	+	–
	PH	++	++	+	++		++	++		+
Delipidated LDL As.	IEP, ID	++	+	–	+	+	++	–	+	–
	PH	++	++	+	++		++	+		+
Maleyl LDL AS.	IEP, ID	+	+	+	+	+	+	–	+	–
	PH	++	++	+	++		++	+		+
Maleyl apo-B AS.	IEP, ID	+	+	+	+	+	+	–	+	–
	PH	++	+	+	++		++	++		+
VLDL AS.	IEP, ID	++	+	–	–	–	++	–	++	+
	PH	++	+	+	++		++	+		++
VLDL apoB AS.	IEP, ID	+	+	–	–	–	+	+	++	+
	PH	+	+	+	++		++	++		+
VLDL apoC AS.	IEP, ID	–	+	–	+	+	+	–	+	++
	PH	+	–	+	++		++	+		++

ID: 10–30 μg antigen; 15–25 μl antiserum.
IEP: 10–30 μg antigen; 100 μl antiserum.
PH: 1–2 μg antigen; 50 μl antiserum, dilution 1/2 to 1/512.

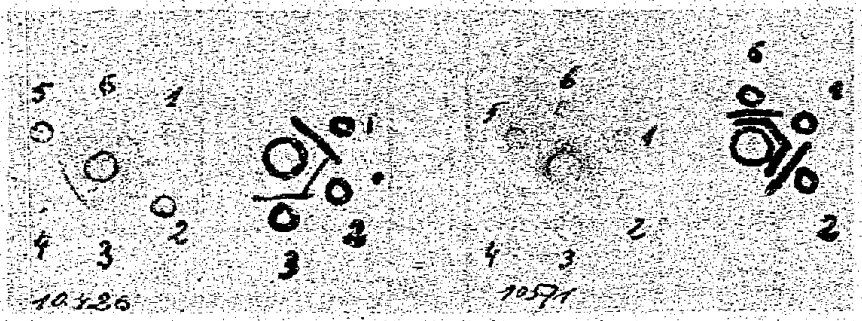

Fig. 4. ID: Slide I: Antiserum against Apo B of VLDL: 1, Apo. B of VLDL; 2, 5, Apo. C; 3, 6, Mal. LDL; 4, VLDL. Slide II: Antiserum against Apo. C: 1, Mal. Apo. B; 2, 4, 6, Apo. C; 3, LDL; 4, Apo B of VLDL.

4. Discussion

The use of gel precipitation tests and of the very sensitive micro-haemagglutination technique allowed to establish a comparison between maleylated LDL and its delipidation products.

If maleylation and partial delipidation did affect but little the immunological reactivity of LDL, total delipidation considerably altered it; immunogenicity and micropassive haemagglutination were still positive, but ID and IEP became negative.

The restoration of the gel precipitation reactivity of Apo B by SDS in absence of the lipid moiety, could be explained by a conformational reorganization of the molecule and the subsequent precipitation properties. Simultaneously exteriorization of hidden antigenic determinants, results in the appearance of several precipitation lines.

Another aspect of this investigation was the interpretation of the intriguing results we found both in gel precipitation and passive haemagglutination, when Apo C and Apo B reacted with their homologous and respectively heterologous antisera. Three main hypothesis are suggested:

– Contamination of Apo C by Apo B and inversly, during their preparation. The two methods and the repeated washings used for their separation, scarcely allow this supposition.

– The presence of Apo C all over the density range 1.006 to 1.045. This fact already described by Alaupovic and al. [8], and also admitted by Gotto and al. [9] would have been even more consistent if we would have found two distinct precipitation lines all over these density ranges, with both Apo C and Apo B antisera.

– The existence of a cross reaction Apo C–Apo B and vice versa, could be also considered as an expression of a similarity limited to a restrained region of both Apo B and Apo C molecules. The partial relationship and the spur that have been noted in ID, rather lend support to this interpretation.

Acknowledgements

This study was supported by the Institut National de la Santé et de la Recherche Médicale and by a grant from the Fondation pour la Recherche Médicale Française.

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